



**NTP**  
National Toxicology Program

## **Benzene ADME in Genetically Diverse Mouse Strains**

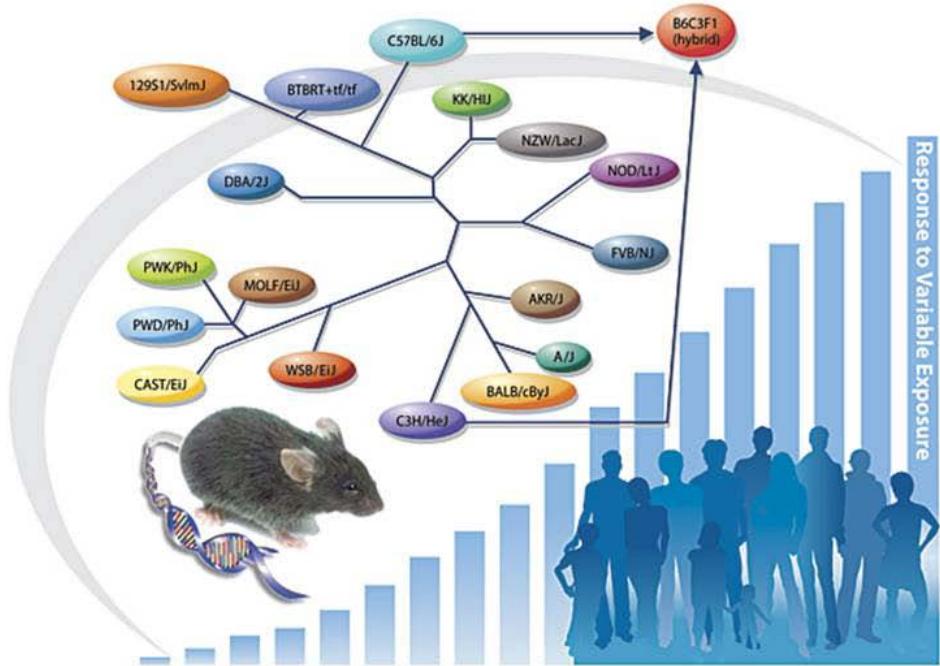
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National Institute of Environmental Health Sciences

NTP Board of Scientific Counselors  
December 9-10, 2009





## HOST SUSCEPTIBILITY BRANCH





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## Tier 1 strains: Benzene Metabolic Diversity Studies

Use Rational	Strain
Genome sequence is publicly available, accounts for >85% of inbred strains in research	129S1/SvImJ A/J AKR/J B6C3F1 BALB/cByJ C3H/HeJ C57bl/6j DBA/2J FVB/NJ NOD/LtJ
Derived from major taxonomic groups	CAST/EiJ ( <i>M. m. castaneus</i> ) PWD/PhJ ( <i>M. m. musculus</i> ) WS B/EiJ ( <i>M. m. domesticus</i> ) MOLF/EiJ ( <i>M. m. molossinus</i> )
Strain used in behavioral studies	BTBR T+ tf/J
Distantly related to other inbred strains	KK/Hij
Strain used in immunological studies	NZW/LacJ

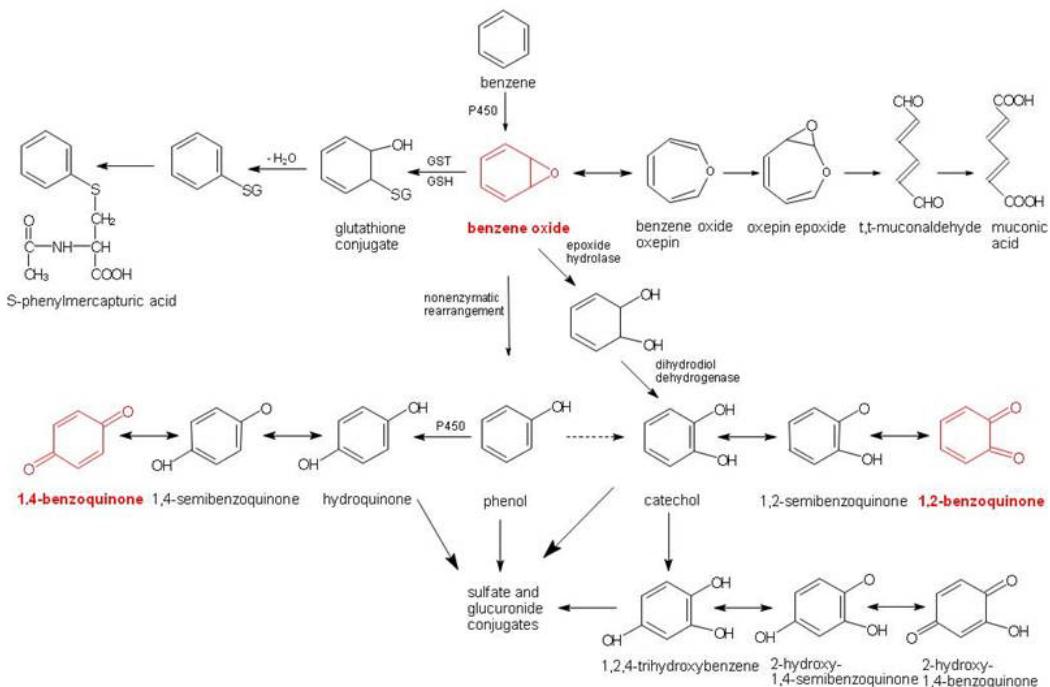


## Rationale and Background

- Benzene - human and rodent carcinogen
- ADME kinetics - significant differences in toxicokinetics that may correlate with toxicity
- The MOA of benzene-induced hematotoxicity and genotoxicity remain unclear
- Toxicity and cytogenetic alterations were observed in myeloid progenitor cells

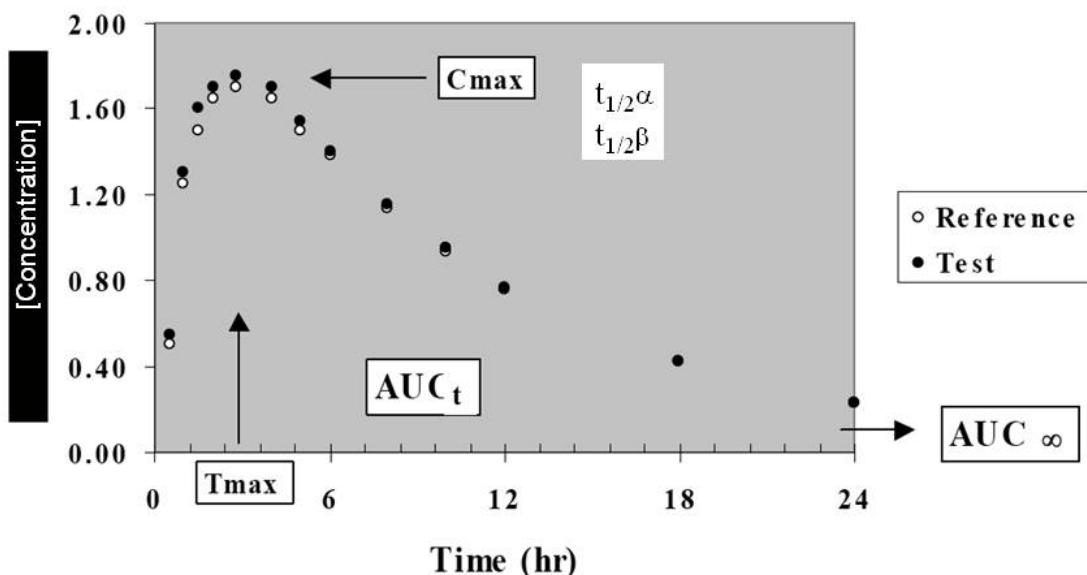


## Benzene Metabolism





## Mean Reference and Test Results

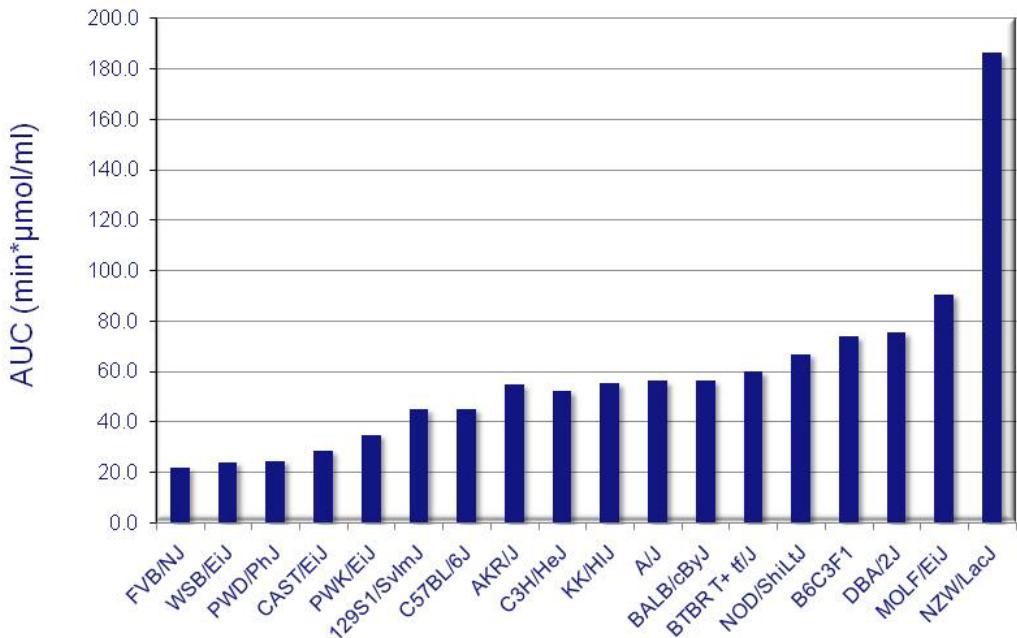


**Kinetic Parameters in Blood Following Oral Administration in Male Mice**

Strain	AUC min* µmol/ml	C <sub>max</sub> nmol/ml	T <sub>max</sub> min	t <sub>1/2?</sub> min	t <sub>1/2?</sub> min	CL_F ml/min
NZW/LacJ	186	1360	30	8.8	71	0.1
MOLF/EiJ	90	229	3	0.3	272	0.1
DBA/2J	75	313	10	1.4	160	0.4
B6C3F1	74	294	14	2.2	165	0.3
NOD/ShiLtJ	67	509	4	0.5	88	0.5
BTBR T+ tf/J	60	356	11	1.9	108	0.7
BALB/cByJ	56	402	10	1.6	90	0.5
A/J	56	232	3	0.3	166	0.4
KK/Hij	55	749	4	0.7	48	0.6
AKR/J	55	389	18	4.0	84	0.6
C3H/HeJ	54	307	15	2.8	112	0.4
<b>C57BL/6J</b>	<b>45</b>	<b>359</b>	<b>17</b>	<b>3.9</b>	<b>74</b>	<b>0.6</b>
129S1/SvImJ	45	267	15	2.7	106	0.5
PWK/EiJ	35	272	11	1.9	81	0.4
CAST/EiJ	28	226	17	3.8	74	0.8
PWD/PhJ	25	431	11	2.9	31	1.0
WSB/EiJ	24	197	21	5.1	68	0.5
FVB/NJ	22	255	16	4.0	47	0.8

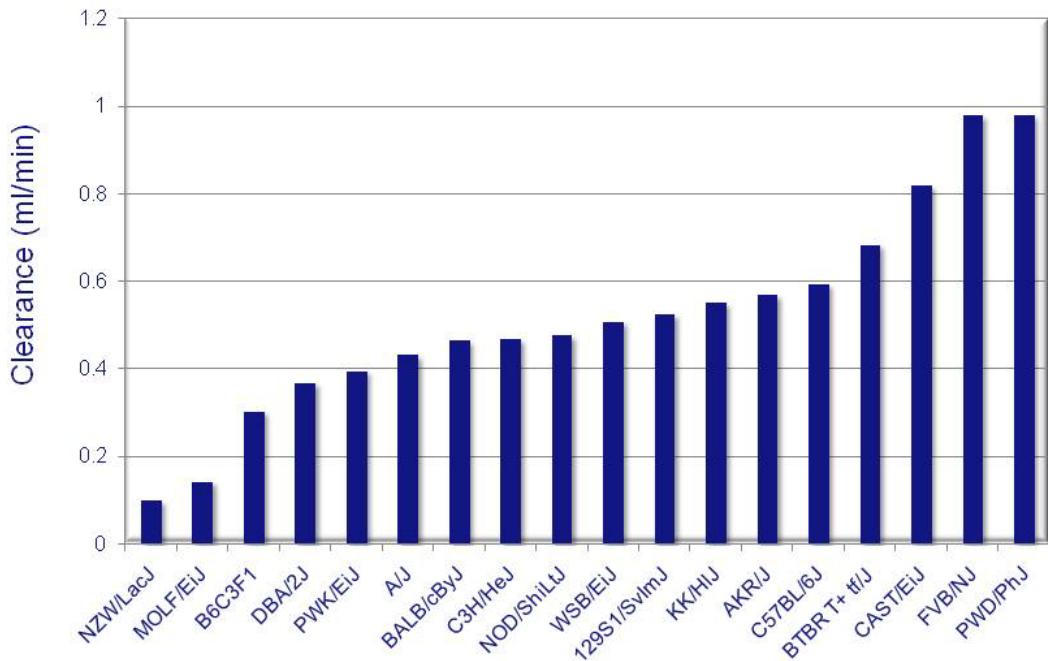


**Rank Order [<sup>14</sup>C] Benzene Equivalents AUC (min\* $\mu$ mol/ml)  
Following a Single Oral Exposure (100  $\mu$ g/kg)**





**Rank Order [<sup>14</sup>C] Benzene Equivalents Clearance (ml/min)  
Following a Single Oral Exposure (100 µg/kg)**



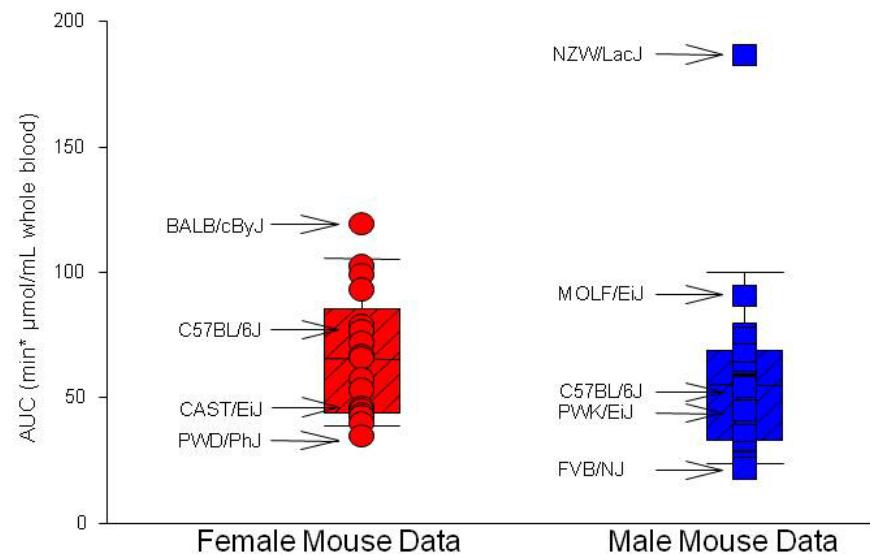


## Kinetic Parameters in Blood Following Oral Administration in Female Mice

Strain	AUC min* µmol/ml	C <sub>max</sub> nmol/ml	T <sub>max</sub> min	t <sub>1/2?</sub> min	t <sub>1/2?</sub> min	CL_F ml/min
BALB/cByJ	119	519	25	5.0	141	0.24
C3H/HeJ	102	472	11	1.8	142	0.23
AKR/J	99	424	36	8.4	134	0.38
A/J	93	239	33	6.0	245	0.21
NZW/LacJ	78	423	31	7.7	104	0.24
<b>C57BL/6J</b>	<b>76</b>	<b>603</b>	<b>19</b>	<b>4.4</b>	<b>74</b>	<b>0.30</b>
KK/HIJ	72	361	30	7.1	114	0.31
B6C3F1	67	545	14	2.8	74	0.32
BTBR T+ tf/J	65	395	32	8.5	90	0.48
FVB/NJ	57	514	32	11.9	49	0.35
DBA/2J	53	199	17	2.8	172	0.23
129S1/SvImJ	46	402	33	12.4	50	0.50
CAST/EiJ	45	194	20	3.6	145	0.18
NOD/ShiLtJ	43	289	25	6.3	84	0.29
WSB/EiJ	42	315	20	4.7	77	0.38
PWK/EiJ	40	434	11	2.2	56	0.33
PWD/PhJ	34	371	12	2.6	55	0.39
MOLF/EiJ	tbd	tbd	tbd	tbd	tbd	tbd



## Distribution of AUC<sub>blood</sub> Values for Female and Male Mice

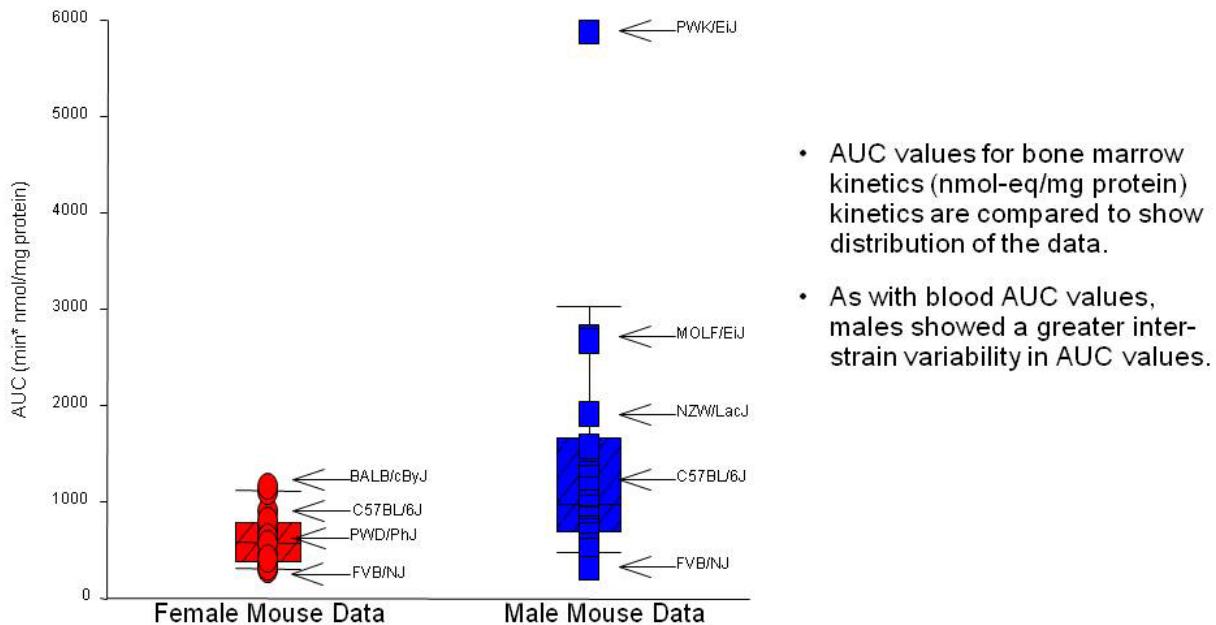


**Kinetic Parameters in Bone Marrow Following Oral Administration in Male Mice**

Strain	AUC min*nmol/mg protein	Cmax nmol/mg	Tmax min	t1/2? min	t1/2? min	CL_F mg/min
PWK/EiJ	5880	3.71	30	3.7	1078	2.3
MOLF/EiJ	2713	5.68	37	6.5	304	4.7
B6C3F1	2667	5.46	72	16.7	284	8.4
NZW/LacJ	1911	8.75	65	24.7	94	12.1
KK/Hij	1577	9.36	36	10.4	88	19.3
NOD/ShiLtJ	1248	4.35	27	5.2	179	25.6
129S1/SvImJ	1130	7.48	33	9.4	78	21.0
BALB/cByJ	998	4.21	32	7.1	140	26.2
DBA/2J	947	4.41	35	8.4	122	29.2
AKR/J	834	3.16	31	6.4	160	37.4
<b>C57BL/6J</b>	<b>823</b>	<b>2.47</b>	<b>37</b>	<b>7.4</b>	<b>204</b>	<b>32.5</b>
CAST/EiJ	724	4.43	34	9.3	87	24.2
A/J	696	3.85	67	46.6	46	34.7
WSB/EiJ	663	2.92	53	16.3	114	18.3
C3H/HeJ	550	3.46	58	40.5	41	44.4
BTBR T+ tf/J	492	3.17	40	12.9	75	83.2
FVB/NJ	<b>315</b>	2.32	42	17.2	56	55.3
PWD/PhJ	1286	8.20	27	6.8	88	18.7

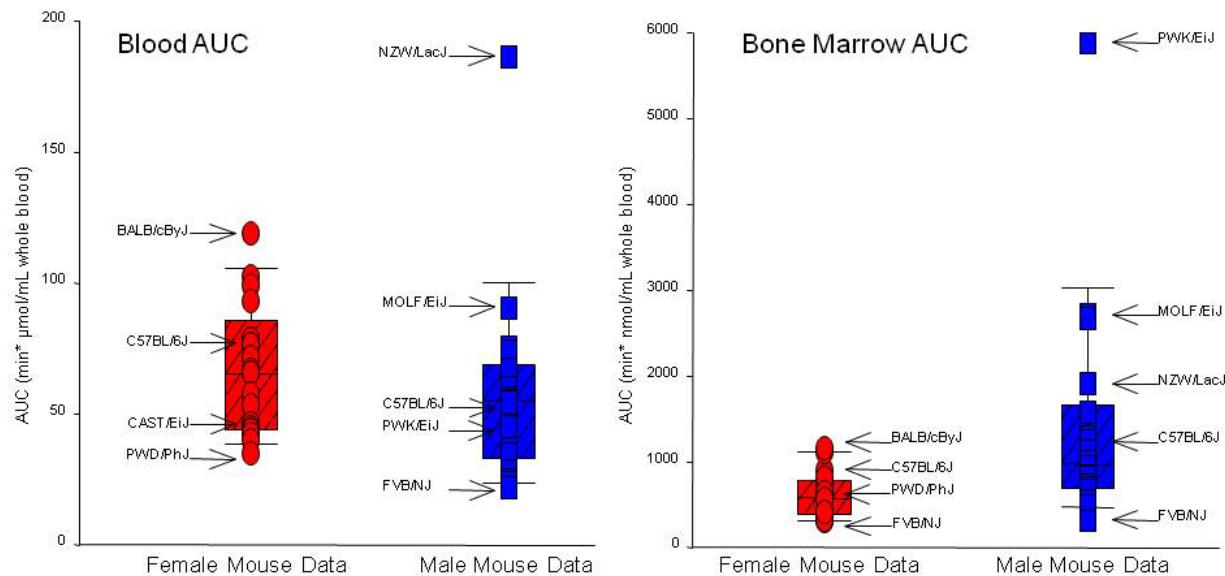


## Distribution of AUC<sub>marrow</sub> Values for Female and Male Mice





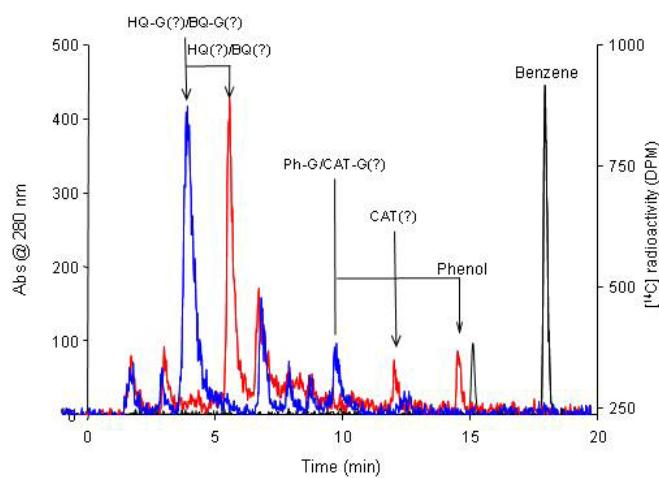
## Distribution of AUC<sub>blood</sub> & AUC<sub>marrow</sub> Values for Female and Male Mice



- AUC values for blood and bone marrow kinetics appear to be normally distributed for most strains
- Outlier AUC values were somewhat consistent between blood and bone marrow.



## HPLC-Radiometric Urinalysis – C57BL/6J Male Mice

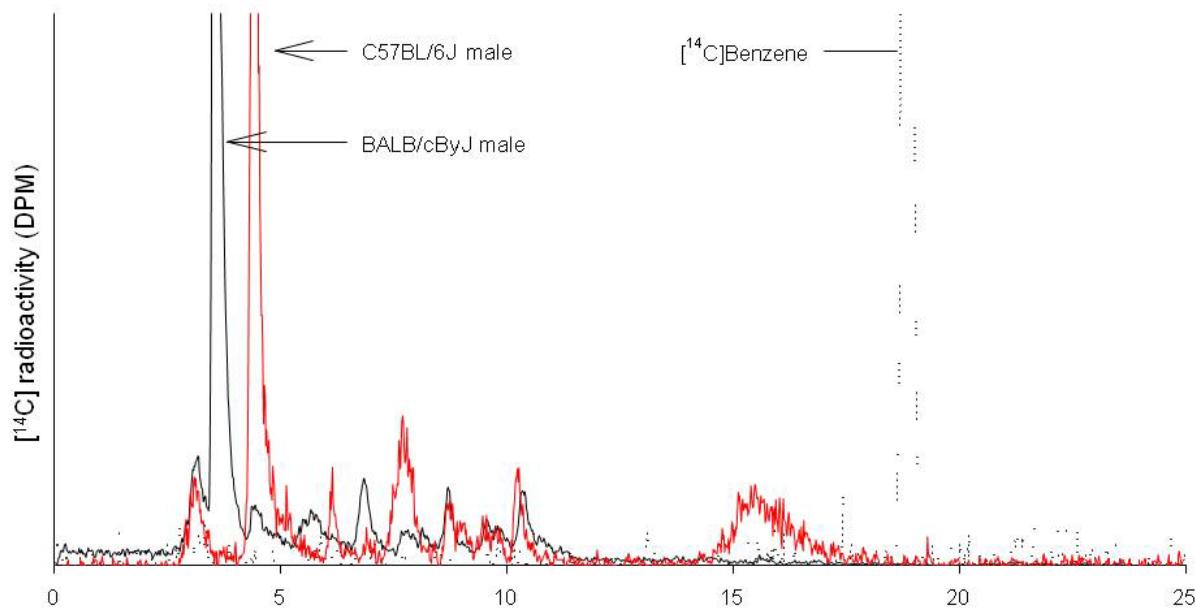


### Chromatography of standard water-soluble benzene metabolites

- BQ-G: benzoquinone-glucuronide;
- HQ-G: hydroquinone-glucuronide;
- Ph-G: phenyl-glucuronide;
- CAT-G: catechol-glucuronide;
- HQ-S: hydroquinone-monosulfate;
- Ph-MA: phenyl-mercapturic acid;
- P-S: phenyl-sulfate;
- CAT-S: catechol-sulfate (Sabourin, 1988)



## BALB/cByJ Male Mice Appear to Generate a Novel Metabolite that is Excreted in Urine





## Summary

- These data aid the Host Susceptibility Initiative in selection of NTP animal model systems for future research projects (28-day inhalation study for benzene; additional chemicals)
- Proof-of-Principle study with benzene shows 10-fold or greater differences in ADME kinetics between strains in males but less in females
- Additional information obtained from ADME in genetically diverse strains is significant for understanding mechanisms of toxicity (novel metabolite(s)) that may reflect human genetic diversity



## Future Directions

- Using the NTP-Perlegen genetic sequence data base of these strains, HSB scientists and collaborators can statistically correlate genome-wide SNP differences with quantitative measures for each strain-specific ADME trait and identify quantitative trait loci or QTL and the candidate gene allelic variants (next speaker - Dr. French)
- Bioinformatics and functional analysis of the mouse candidate gene allelic variants may then be used to identify human orthologs, which will aid in extrapolation of ADME and toxicity data across species and support functional validation studies in mouse and human cells and tissues.



National Toxicology Program

## Acknowledgements

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